

**COMPARISON OF JUNGLE GREENS (KALAVAI KERALI) WITH
WITH AMARANTH (~~AMARANTHUS GAMBENSIS~~) FOR
CALCIUM ~~DEFICIENCY~~ BY ALBINO RATS ON
"POOR RICE DIET".**

By

Mary Vasanthi Kusari. Devadoss.

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I. INTRODUCTION.

The low nutritive value of the Indian rice diets is attributed to the almost complete omission of the protective foods such as green leafy vegetables, fruits and animal foods in the diet. When foods rich in calcium like milk are consumed in little or no quantities as indicated by Aykroyd *et al* (1937)¹, calcium which is one of the most important elements required by the body, is absent in the diets. Therefore, green leafy vegetables, an inexpensive and readily available source of calcium, next to milk in calcium content, can play an important role in the poor Indian diet.

The fact that the poor vegetarian diets are mainly based on cereals, with the inclusion of small quantities of certain pulses, vegetable oils, negligible amount of milk and other protective foods, has been reported by Subramanyam *et al* (1949)², Murthy *et al* (1955)³, Subramanyam (1956)⁴ and Mc Carrison (1956)⁵. These authors have further pointed out that the poor vegetarian rice diets are deficient not only in calcium and iron, but also in Vitamin A, vitamin B complex and proteins of high biological value.

Aykroyd *et al* (1937)¹ have demonstrated through

animal experiments that ~~supplementation~~ of the rice diets with calcium in the form of calcium ~~sulfate~~ or lactate, resulted in considerable increase in the growth promoting value of the poor vegetarian rice diets, indicating thereby that calcium is one of the crucial limiting deficiencies in such diets. The importance of calcium is supported by ~~Bentley's~~ (1956)⁶ work on the poor Indian rice diets. He ^{has} showed that ~~supplementation~~ of such diets with protein isolates of soya bean, bengal gram or ~~groundnut~~, did not result in any significant increase in the growth promoting value of the diets, until and unless the calcium deficiency was also made up simultaneously.

Among all the mineral elements, calcium is present in the body in the highest amount, and therefore Irving (1957)⁷ considers it as the most important inorganic element in the body. According to Best (1961)⁸ calcium is an indispensable mineral constituent of fluid and solid tissues of all animal bodies, and plays an important part in a variety of physiological ~~processes~~.

The dietary requirement of calcium at different stages of the human life cycle namely infancy, childhood, adulthood, old age and special conditions such as pregnancy and lactation, have been published by the National Research Council of U.S.A., (1958)⁹, The Food and Agricultural Organization of the United Nations (1962)¹⁰ and the Nutrition Advisory Committee of the Indian Council of Medical Research

(Aykroyd 1962)¹¹. In all these ~~recommendations~~, the allowances of calcium for children range from 1 gm. to 1.5 gm. per day, which is greater than the ~~requirements~~ for the normal adult. This ~~increased~~ ~~requirements~~ is suggested because of the greater demands of growing children for this element. Bariseman et al (1959)¹² point out, that when the dietary calcium level is decreased, some of the ~~manifestations~~ of the calcium ~~deficiencies~~ such as ~~irritability~~, loss of appetite, ~~constipation~~, rickets in children, and ~~osteomalacia~~ in adults occur.

In order to find out the existing ~~nutritional~~ status of rural people, as part of the Community Nutrition project, a diet survey was carried out by the ~~investigator~~ in the village of ~~Bariseman~~ in ~~Bariseman~~ Block, ~~Bariseman~~ District, in 1962, where 160 families were ~~interviewed~~. The results showed that the children in that village received only about 0.2 to 0.3 gm. of calcium per day per person, and as a result of this low intake, 69 per cent of the children were found to be suffering from rickets and bow legs, as shown by the clinical examinations by a physician. ~~Bariseman~~

This low intake of calcium was due to the fact, that soon after weaning, the children were ~~introduced~~ to the diet of adults, which were highly starchy ~~consisting~~ of 96 per cent of cereals, two percent dhal, one per cent greens, and one percent ~~vegetables~~ such as brinjals and pumpkin. Since 81 per cent of the people in this village were on the low

economic status with an average per capita income of 75 rupees per day, they could not include milk in their dietaries. For those few who could afford to buy milk, it was not available. Hence, they had to depend on some other inexpensive alternatives such as green leafy vegetables as a source of calcium. Fortunately there were many varieties of wild leafy vegetables found among the weeds in the fields, available throughout the year. The people in the village were familiar with these leafy vegetables, and consumed them in the a mixed form, popularly known as kalavai keerai. However, the amount of kalavai keerai included in their dietaries was as low as half an ounce per person per week, which furnished negligible amounts of calcium. Investigations on their methods of cooking also revealed, that because they cooked the greens in excess water and discarded the cooking water, the availability of calcium from the greens was further reduced, since according to Irving (1957)⁷ the only cooking procedure which affects the final calcium content of cooked foods is boiling.

Irving (1957)⁷ has shown, that next to milk, green leafy vegetables form the best sources of calcium. Subrahmanyan (1949)² points out that cheap and easily available food materials such as green leafy vegetables can supplement the poor rice diet, thus offering more scope for a practical approach to the food problems not only in India, but also in

other parts of the world which have similar difficulties.

While nature has abundantly blessed the rural areas with perennial supplies of ~~varieties~~ of kalavai keerai, the nutritive value of such mixtures has not been studied so far. Some attempts were made by ~~Theophilus et al~~ Theophilus et al (1949)¹³ to analyse individually the nutrient content of some selected wild greens such as Kuppameni (*Achypha indica*) ~~Eluvattai~~ (*Buchavia repens*), ~~Puchattai~~ keerai (*Euphorbia hirta*), Vella keerai (*Cleome viscosa*), Herringi (*Tribulus terrestris*), Saravalli keerai (*Trianthema amagosa*), Punnaku-keerai (*Corchorus aestivus*) and Kuppai keerai (*Amaranthus viridis*), but not in the form of the mixture as consumed in the villages.

The present experiment was therefore conducted to study the nutrient content of the kalavai keerai as a mixture, and to find out its effects on calcium retention in albino rats fed on "Poor Rice Diet" as the basal diet. The poor rice diet, according to Aykroyd et al (1937)¹ represents the dietary pattern of the poor South Indians.

The ~~availability~~ availability of calcium for growing albino rats from kalavai keerai ~~supplemented~~ supplemented Poor Rice Diet was compared with that of amaranth, the well known and commonly

* The "Poor Rice Diet" is the accepted terminology of a diet formulated by Mason and Theophilus (1945)¹⁴ and its composition is given in Table IV on page 55.

consumed leafy vegetable, keeping the calcium levels identical at 50 mgm. per day.

It is hoped that the findings of this study will be useful to offer an inexpensive source of calcium in the dietaries of the poor, and a better source of calcium than amaranth in the dietaries of the rich, and stimulate further research on the numerous varieties of wild greens.

The criteria used for judging the availability of calcium were growth, calcium balance and calcium in bone ash.

II. REVIEW OF LITERATURES.

The review of literature is discussed on the following aspects.

- A. Importance of calcium in nutrition.
- B. Factors affecting calcium balance.
- C. Methods of assessing the availability of calcium.
- D. Nutritive value of leafy vegetables.

A. Importance of Calcium in Nutrition:

Gilbert (1948)¹⁵ points out that calcium is one of the important biological mineral elements, and is necessary for the growth of all animals and all green plants with the exception of some lower algae. Moore (1952)¹⁶ states that electrolytes such as calcium, potassium and sodium are very important for the proper functioning of proteins.

Sherman (1936)¹⁷, Sussman (1937)¹⁸, Mc Carrison (1956)⁵, Maynard (1956)¹⁹, and Davidson et al (1959)¹² have reported that calcium is an outstanding element of the mineral matter of skeleton. It is an important constituent of the bones and teeth. It gives rigidity and strength to the skeletal structures and it is the most abundant element in the body, forming 98 to 99 per cent of the mineral content of the skeletal system. The Dairy Council

Digest (1960)²⁰ points out that calcium is an essential nutrient with multiple functions. West et al (1961)²¹ claim that without bone development there cannot be growth, and since calcium is intimately related to bone formation, it is considered to be essential for growth.

The part played by calcium in the regulation of body processes has been a subject of interest for many workers. Beuwerke (1948)²² reviews the importance of calcium in the following words, "Calcium is an important constituent of the skeletal structure, an aid in the control of the contractility of the muscles, (particularly cardiac rhythmicity) the preservation of normal response of numerous tissues to stimuli, the normal coagulability of the blood and as an important regulator, in the control of membrane permeability." Gilbert (1948)¹⁵ states, "In animals calcium is the outstanding single constituent of bones and teeth, and the normal rhythm of the heart muscles, the excitability of other muscles and nerves, depend on their being constantly bathed in lymph, containing physiologically normal amount of calcium. The clotting of blood and the regulation of the permeability of the membrane also depend on a constant maintenance of this element." Meltzer (1952)²³ reports "Calcium is capable of correcting many disturbances of equilibrium in the body whatever the directions of the deviation, from the normal may be, and any abnormal effect which sodium, potassium

or magnesium may produce, calcium is capable of re-establishing the normal equilibrium." The important functions of calcium are highlighted by Sherman (1952)²⁴ as the maintenance of the right degree of firmness in protoplasm and the soft tissues. Even though calcium occurs in blood in very minute quantities it is absolutely essential for the normal action of the heart muscles. Maynard (1956)¹⁹ reports that calcium as a soluble salt, helps to maintain the right degrees of osmotic relations and acid base balance. Kleiner (1958)²⁵ points out, that calcium is needed by all the cells and that it is one of the important ions required for the physiological balance and the nervous tissues. He further states that if the blood calcium level falls below the normal requirement, it tends to develop hyper irritability and tetany. According to West (1961)²¹ calcium ion is directly related to muscle contraction, and in the absence of calcium, all types of muscles lose their ability to contract.

The role of calcium in nutrition has been demonstrated by Aykroyd *et al* (1938)²⁶ who have shown, that when Nursery School children were given calcium salts as supplements, an ~~enormous~~ increase in weight and growth resulted. This has been supported by Pal and Singh (1938)²⁷. Their studies prove that the nutritive value of South Indian rice diet was enhanced by the addition of basic calcium phosphate as evidenced by the increase in the weight and length of body and femur, and an efficient calcification of bones.

Sherman and Campbell (1935)²⁸ point out, that "The enrichment of calcium intake was followed by a more efficient utilization of the food better growth, earlier maturity. Several indications of higher adult vitality, a large period between the attainment of maturity and the onset of senility, and in less degree an increase in the average length of adult life or life expectation of the adult. In as much as the infant mortality was decreased by the increase in calcium intake, the life expectation at birth was increased in greater degree, than the life expectation of the adult." Toepfer et al (1936)²⁹ report that increasing the calcium content of an adequate diet resulted in more rapid growth with greater economy in the use of food. The rate of normal calcification as shown in young of the second generation was also distinctly increased. Campbell et al (1945)³⁰ stress, that "Higher calcium intake appeared necessary for optimal results and may perhaps have an added interest in relation to the long standing hypothesis that calcium in addition to its specific functions may also exert a general regulatory or stabilizing effect." The National Research Council of U.S.A., (1958)⁹ reports, that increased intake of calcium lengthens life span and improves the reproductive and lactational performances of rats.

In addition to all these functions, Maynard (1936)¹⁹ and West (1961)²¹ point out that calcium activates a number of enzymes such as lipase, succinic dehydrogenase, adenosine-triphosphate and certain proteolytic enzymes.

Factors Affecting Calcium Balance:

The factors affecting calcium balance are:

1. Calcium absorption, and
2. Regulation of calcium balance.

1. Calcium absorption:

The absorption and utilization of calcium in the body are affected by, a) Several important dietary factors such as: phytic acid, phosphates, fibre content of food, galactose, oxalic acid, protein, fats and fatty acids, citric acid, lactose, vitamin D, and the relative level of calcium and phosphorus in the diet and b) Reaction of intestinal contents.

a. Dietary factors: The dietary factors which inhibit calcium absorption are phytic acid, phosphates, fibre content of food, galactose, and oxalic acid; while factors such as protein, fats and fatty acids, citric acid, lactose, vitamin D and the relative level of calcium and phosphorus in the diet, favour calcium absorption.

Phytic acid: Nicholls (1945)³¹, Brine *et al* (1955)³², Albright (1956)³³, Davidson *et al* (1959)¹² and Subramanyam *et al* (1961)³⁴ report that phytic acid (inositol hexa-phosphoric acid) which occurs as a major part of the organic phosphorus content of cereals, partly in the free state and partly as

phytin, forms an insoluble salt with calcium and thus interferes with calcium absorption.

Phosphates: Leichsenring *et al* (1951)³⁵ have reported a marked depression of calcium retention when phosphate was added to the diet. The possibility that dietary calcium may combine with phosphorus and form an insoluble salt which would be poorly absorbed, has been suggested by Brine *et al* (1955)³². Davidson *et al* (1959)¹² point out, that before the discovery of phytic acid, it was generally believed that the total phosphate intake had a crucial influence on calcium absorption. Subrahmanyam *et al* (1961)³⁴ conclude that excess phosphorus in the diet associated with a low dietary calcium greatly inhibits calcium absorption, causing typical low calcium rickets.

Fibre content of food: Johnson (1961)³⁶ states from a study of three patients with ileostomies and colostomies, that the normal reaction of the ileum is acid, but an excess of fibre in the diet changed the reaction to neutral, which possibly lowered calcium absorption.

Galactose: Heggenes (1960)³⁷ states that animals fed ^{on} galactose gained weight slowly in comparison with those fed ^{on} glucose, and absorbed slightly more calcium and excreted significantly more calcium in the urine. The author stresses that feeding

galactose results in a negative calcium balance and presumably results in demineralisation of the skeleton.

Oxalic acid: Giri (1940)³⁸, Johnston et al (1952)³⁹, Brine et al (1955)³², Maynard (1956)¹⁹, Davidson et al (1959)¹² and Subrahmanyam et al (1961)³⁴ state that some natural foods such as spinach, beet greens and cocoa contain appreciable amounts of soluble oxalates which react with calcium and form insoluble calcium oxalate, which may inhibit the absorption of calcium.

Protein: Many workers have claimed that there is an association between protein and calcium in the diet; calcium absorption and utilisation being better with high protein intakes. Conner et al (1936)⁴⁰ report that the total body calcium in rats increase during growth by increased protein in the diet. Mc Cance et al (1957)⁴¹ have proved that calcium absorption is dependent on the protein intake, and that such absorption may fall to very low levels in the absence of dietary protein, according to Brine et al (1955)³² protein promotes the absorption of calcium. Davidson et al (1959)¹² explain that amino acids liberated in the course of protein digestion probably form very soluble calcium salts which are easily available to the cells.

Fats and fatty acids: Basu and Nath (1946)⁴² report that inclusion of mustard, sesame, groundnut oil or butter fat in

the diet of four human subjects, greatly favour the absorption of calcium and phosphorus, and the utilization of minerals is better than in the absence of added fat. With coconut oil however, the elimination of calcium through the faeces was greatly increased; the faecal calcium being in three cases greater than the amount of dietary calcium. Rao *et al* (1952)⁴³ report that fat plays a fundamental role in influencing the mineral metabolism, which is of considerable nutritional significance during the growth period. The author concludes that moderate amounts of fat in the diet favour the utilization of calcium. The fact that fats make the contents of intestines more acid and thereby increase the absorption of calcium and phosphorus has been noted by Boyd *et al* (1957)⁴⁴. Irving (1957)⁷ states that unabsorbed split fat in the intestines will form insoluble calcium soaps, and thus may interfere with calcium absorption. Such conditions may happen when the flow of bile is obstructed. French (1957)⁴⁵ points out that utilization of calcium fell consistently as the fat content of the diet was raised above five per cent. Subrahmanyam (1961)³⁴ points out that faulty absorption of fats leading to large amounts of free fatty acids in the stools may result in considerable losses of calcium in the faeces.

~~Other work:~~ Landford (1939)⁴⁶ records an average increase of eight per cent calcium retention in albino rats when their diet was supplemented with five ml. of Florida non-Palencia

orange juice per rat per day. Mallon et al (1946)⁴⁷ noted seven per cent increased calcium retention upon supplementing five ml. of grape fruit juice to each growing albino rat per day, but neither increased nor decreased calcium retention was noticed when five ml. of tomato juice was given to growing albino rats. Mallon (1952)⁴⁸ demonstrated the beneficial effect of citric acid upon calcium absorption by conducting experiments on growing female albino rats.

Lactose: Mitchell et al (1961)⁴⁹ observe that lactose not only increases the retention of calcium but also increases the bone ash, body ash and body calcium. Bergain (1961)⁵⁰ also attributes the beneficial effect of lactose to an increased acidity of the intestinal contents which result in increased calcium absorption. Vaughan and Filer (1960)⁵¹ on the basis of their studies formulate several theories on the beneficial effects of lactose on calcium retention as follows: "1. Lactose lowers the pH of the gut, by promoting fermentation. 2. Some sugars influence the bone cells in the process of ossification, and 3. Lactose and other sugars may cause a stimulation of the flow of digestive juices including bile, which in turn may augment calcium absorption."

Vitamin D: According to Nicholls (1945)⁵¹ vitamin D is a necessity for the proper absorption of calcium from the alimentary tract and its maintenance in normal amounts in the blood.

Beuwkes and Adelia (1948)²² state that calcium requires vitamin D for its optimal utilization. Henschkyen (1961)⁵² has proved that vitamin D is necessary for the intestinal absorption of calcium and for normal bone deposition, by promoting normal bone development either by increasing calcium and phosphorus absorption, or by promoting calcium and phosphorus metabolism after absorption. Stribanjan (1961)⁵⁴ shows that vitamin D occupies a dominant role in the metabolism of calcium.

The relative level of calcium and phosphorus in the diet: Nicholls (1945)³¹ and Albright (1956)³³ report that the relative and absolute levels of calcium and phosphorus in the diet influence the absorption of calcium.

6. Reaction of the intestinal contents: McGowan (1957)⁵³ on the basis of balance experiments has stressed the importance of the gastric hydrochloric acid in the absorption of calcium, and considered it the chief single factor in this process. Busse-berger (1957)⁵⁴ reports that gastritis in puppies caused severe osteoporosis and even spontaneous fractures. Irving (1957)⁷ states, "The addition of acid to the diet or giving an acidic type of diet may apparently increase calcium absorption, and alkali in the diet may have the reverse effect. However, while intestinal absorption may be improved by giving acids, in many cases the urinary excretion of calcium is also increased, causing in the end a greater total loss of calcium

from the body. On the other hand it has been stated that the reaction of the diet has no effect on calcium absorption, and that alkaline solutions introduced into the intestine rapidly become acid. Similarly it has been stated that acid solutions actually depress calcium absorption. It seems almost impossible to draw any conclusions from the very contradictory evidence that exists." According to Subrahmanyan (1961)³⁴ the pH of gastro intestinal tract influence the absorption of calcium since the solubility and ionisation of most calcium salts increases with acidity.

2. Regulation of calcium balance:

Subrahmanyan (1961)³⁴ points out that the amount of calcium in the body is the result of a finely adjusted balance between intake and output, which in turn is determined by a further balance that exists between the amount of calcium in the fluids of the body and the longer reserves in the calcium bank of the skeleton. Albright et al (1959)⁵⁵ list three factors, namely parathyroid gland, vitamin D and kidneys, as helping in the regulation of calcium balance.

Parathyroid gland: Albright et al (1959)⁵⁵ noticed that parathyroid gland influences the calcium level in the blood. They point out that in rare instances where the glands are over active, the osteoclasts are stimulated to excessive activity, with the result that calcium is withdrawn from the bones, and

the amount of ionized calcium in the blood rises accordingly.

Vitamin D: According to Albright *et al* (1959)⁵⁵ vitamin D has a direct effect on raising the calcium content by causing resorption of bone, thus ~~duplication~~ some of the effects of ~~parathyroid~~ hormone.

Kidneys: Albright *et al* (1959)⁵⁵ report, "Kidneys help in the maintenance of calcium balance by ~~eliminating~~ the excess present in the body fluids, and if they fail in their activities, osteomalacia would appear, due to defective tubular reabsorption of calcium through renal tubules."

d. Methods of assessing the availability of Calcium:

The different methods used for assessing the availability of calcium are:-

1. Calcium balance studies,
2. Calcium content of blood,
3. Calcium content of bones by
 - a) ashing method,
 - b) microscopic method.
4. Calcium content of cartilage.

Calcium Balance Studies:

The determination of the availability of calcium from the food using calcium balance studies as a criterion was followed by many investigators even from the very early

days. Accordingly Sherman and Hawley (1922)⁵⁶ studying the calcium balances of growing children, found that the retention of calcium was more variable, but always less favourable when half of the milk of the diet was replaced by a carefully prepared mixture of vegetables, (carrots and spinach) which provided the same total calcium intake. The calcium of this mixture of vegetables was clearly less well utilized than milk in these experiments, with three to thirteen year old children.

McClugage and Mendal (1948)⁵⁷ found the calcium of carrots and spinach to be poorly utilized by dogs. Rose (1948)⁵⁸ in a calcium balance experiment with healthy young women whose intake was close to the estimated minimum for equilibrium found the calcium of carrots almost as well utilized as that of milk, McLaughlin (1948)⁵⁹ compared the calcium balances of healthy women on a diet in which 73 per cent of the calcium was from spinach and with one in which 79 per cent was from milk. Both diets furnished considerably more calcium than the average requirement for maintenance, so that there was always an equilibrium in calcium retention. While the spinach diet with a slightly higher calcium intake gave a markedly lower retention, McLaughlin concluded that the positive balance demonstrates at least a partial utilization of the calcium of the spinach.

According to Sherman (1952)²⁴ the method which permits

the most direct approach to the question of the amount of calcium or phosphorus needed in human nutrition is that of balance experiments. The author states, "As both calcium and phosphorus after being metabolized in the body are excreted to a considerable extent by way of the intestine, and as the proportions of eliminated calcium or phosphorus leaving the body by way of the two chief paths of output, (the kidneys and the intestines) may vary widely, conclusion regarding bodily need of calcium or of phosphorus should not be attempted except from experiments which include quantitative determinations in both urine and faeces." Hawk et al (1954)⁶⁰ remark, that the balance study entails the analysis of the food, ingested, and the urine and faeces excreted.

Olson (1955)⁶¹ reports that because of the inability to examine the daily changes in the calcium content of bones directly, many investigators have approached the problem of determining the calcium requirement from the study of balance experiments. In children the results of balance study may be interpreted in relation to growth, and development of the centres of ossification. Brock (1961)⁶² is of the opinion, that balance is obtained when the total net excretion, (stools and urine) equals the intake, provided the subject concerned has been on the same diet for a long period and is in a good "static" metabolic state. The author further claims that the net intestinal absorption (intake minus stool calcium) is a measure of the calcium absorbed from food intake plus intestinal secretions.

2. Calcium Content of Blood:

Halse (1958)⁶³ demonstrates the effect of starvation on the reduction in serum calcium level, in old cows. He conducted the experiment on two old cows of low yield, one not pregnant and the other a month after calving. They were starved for two periods of two days with an interval of two days in between. In the first period of starvation, serum ^{calcium} in both cows fell below 7 mg. per cent, but neither showed any clinical disturbances apart from slight sluggishness and a fall of pulse and respiration rates. In the second period of starvation serum calcium fell but slightly, and remained within normal limits. This clearly points out the relation that exists between the dietary intake and serum calcium level.

1. Calcium Content of Bones:

a) Ashing Method:

In order to assess the relation between the calcium intake and calcium deposited in bones which are the store house of the minerals, they were ashed and analysed for their mineral content. Bone ashing method for analysing calcium content was carried out by Morgulis et al (1949)⁶⁴ and Mc Cann et al (1949)⁶⁵. These two investigators adopted the method of ashing the whole skeleton through different procedures while Bethke et al (1923)⁶⁶ and Calverley et al (1949)⁶⁷ followed the method of ashing the femur bone alone.

b) Microscopic Method:

A delicate test for calcium depositing substances has been described by Mc Collum et al (1949)⁶⁸, as follows:-

"A group of young rats is placed on control diet, for 35 to 45 days, or until they begin to lose control of their hind legs. They are then divided into two groups. The control group continues to receive the same diet unchanged, and a test group is given the fault diet plus the substance which is to be tested for the number of days deemed necessary. When a sufficient number of days have elapsed for the test substance to have produced its effects, the animals are killed, and the bones which are to be studied are split longitudinally. The proximal end of the tibia is best for the purpose. One half of the bone is immersed in a dilute silver nitrate solution, and exposed to light. It is then examined in the solution through a binocular microscope for the presence of a newly formed line of calcification in the proliferative cartilage. This line of calcium which looks like the cross section of a honeycomb under a strong magnification is thickened by exposure to light. If this line is present, the test is positive. The line may be visible to the unaided eye, in untreated bones. The results of the examination of the gross specimen should be confirmed by a study of celloidin sections from the other half of the bone or other bones. The new line of calcification may extend completely across the bone or may be incomplete or fragmentary according to the extent of

the ~~deposition~~ of the lime salts induced by the substance which is under examination. Since complete ~~starvation~~ also causes the typical linear deposit of lime salts to appear in the cartilage of rachitic animals, the food intake of both test and control rats must be carefully watched."

4. Calcium Content of Foods:

Pineke et al (1935)⁶⁹ studied the storage of calcium in the animal body, in order to assess the availability of calcium from ^asome typical foods. In this experiment, healthy young rats of the same ~~nutritional~~ history were placed when four weeks old, on a diet in which half of the skimmed milk was replaced by enough dried spinach or dried kale to provide the same amount of calcium. At 60 days of age the animals were killed, and their bodies analysed for calcium. From the findings of this study it was found out, that the calcium of kale, was nearly as well utilized as the calcium of milk, but the calcium of the spinach was utilized very poorly.

Conner et al (1936)⁴⁰ adopted the carcass analysis method in order to assess the relation between the protein intake and the rate of ~~calcification~~. In this method, the animals were ~~killed~~ with chloroform and brushed to remove any food or other foreign particles from the fur. The gastro intestinal tract was dissected out its contents weighed and subtracted from the live weight of the rat to give the net weight, which was used as a basis in all calculations.

Lanford et al (1938)⁷⁰ made use of this method, for studying the calcium content of the body as influenced by that of the food. They remark that although the percentage of body calcium finally reached was the same for the higher levels of intake, the rate of calcification was somewhat greater on the diet providing 0.80 percent than on that with 0.64 per cent of calcium. Speirs (1939)⁷¹ also followed this method and she points out, that a more exact value for measuring calcium utilization is expressed in the calcium utilization factor, which is the rate of the calcium retained to that ingested. The difference between the calcium found by analysis at the end of the experimental period and that calculated to have been present at 28 days, equals the calcium retained.

Lanford et al (1941)⁷² studied the influence of different nutritional conditions upon the normal increase of calcium in the growing body. Mallon et al (1952)⁷³ with the help of this method estimated the effect of orange juice and lime juice, on calcium metabolism. The availability of calcium in three grasses namely cocksfoot, timothy and perennial ryegrass, was studied by Armstrong et al (1958)⁷⁴ by using this method. The experimentation lasted for five weeks and the calcium content in the carcass at the end of the experimental period, was compared with the initial calcium content. Kuppaswamy et al (1959)⁷⁵ also adopted this method in finding the effect of crude, refined and hydrogenated cotton seed oil on calcium metabolism.

Among these methods, the calcium study, and the estimation of calcium in the bones femur and tibia were chosen, due to convenience in operation.

B. Nutritive Value of Leafy Vegetables

The nutritive value of leafy vegetables is considered in the following aspects:

1. General nutritional importance of leafy vegetables.
2. Leafy vegetables as a source of proteins, and
3. Leafy vegetables as a source of minerals.

1. General Nutritional Importance of Leafy Vegetables

Since leafy vegetables are available everywhere at low costs, or even free, it is important to realize their value in nutrition. Mc Gillum *et al* (1959)⁷⁶ discuss the high nutritive value of leafy vegetables as "Leaf is the site of synthesis of protein and carbohydrate and is rich in activity functioning cells for the metabolic processes." Nicholls (1945)⁵¹ states, "Numerous leafy vegetables are in use in the tropics, and with a few exceptions they are wild plants which have received no selective cultivation, hence most of them are fibrous, and coarse flavoured, and in some countries the poorer classes make use of almost any green vegetable provided it is not poisonous." Subrahmanyam *et al* (1949)² suggest, that such leaf cells which contain everything needed for the metabolic processes, could be regarded to be of high nutritive value with respect to all the essential

of an adequate diet. Subrahmanyam *et al* (1949)² experimenting with three generations of albino rats, found that desiccated lucerne leaf had an excellent supplementary value to the poor rice diet on growth, reproduction and lactational performance of the animals; and when amaranth was fed with the poor rice diet as a supplement, it was very useful in correcting the defects of the diets.

The pamphlet 'Green Vegetables for Good Eating' by United States Department of Agriculture (1954)⁷⁷ has pointed out "Green vegetables are important in meals because of the nutrients they contribute, particularly vitamin A and vitamin C". Mc Carrison (1956)⁵ emphasizes that green leafy vegetables form one of the three classes of protective foods, so called because they are rich in mineral elements and vitamins, in which the cereal grains are lacking. By providing the mineral salts to a deficient rice diet, they improve the nutritive value of such grains. He further points out, that the frame work of the leaves which is mainly cellulose, helps in the action of bowels, and adds bulk to the diets. The same point is stressed by Devadas (1959)⁷⁸ who states, that amaranth, agathi, drumstick leaves, menthi leaves, mustard leaves, spinach and many other varieties, are all very rich in minerals, carotene, riboflavin and vitamin C. Singh (1960)⁷⁹ stresses the importance of leafy vegetables as having a very high protective food value. They are rich in calcium, iron, and other minerals

and vitamins A. and C. Besides their soft fibrous nature, they provide the necessary roughage in the diet. The pamphlet 'Why Leafy Vegetables?' by Devadas (1961)⁸⁰ also brings out the importance of leafy vegetables in an emphatic manner, through effective captions such as "Leafy vegetables are a rich storehouse of many food factors" and "Leafy Vegetables help in building strong bones, healthy teeth and gums."

2. Leafy Vegetables as a Source of Proteins:

Deshpande (1954)⁸¹ demonstrated the high biological value of the proteins of leafy vegetables and found them superior to dhal protein. Through animal experiments he has shown, that when amaranth was used at 10 per cent level, the biological value of the leaf protein was 66.8 per cent, whereas for pulses at the same protein level, the biological value was only 50 per cent. He has further shown, that the amino acid composition of amaranth protein suggests the superiority of such protein over that of wheat, rice or other cereals.

Phansalkar et al (1957)⁸² have shown that the inclusion of amaranth as a supplement to provide protein at one per cent level, was found to improve the Protein Efficiency Ratio of various cereals and pulses. Further observations had revealed the fact that when amaranth was used as a partial source of protein in cereal and pulse diets, it had remarkably maintained the normal haemoglobin and plasma concentration in young growing rats. Their ability to regenerate blood protein

was also not much lower than skimmed milk. Sur (1955)⁸³ show that one per cent dehydrated lucerne powder was used efficiently in the treatment of malnutrition in children.

Kamath *et al* (1959)⁸⁴ report that Amaranth and *Trigonella* have been found to be deficient in methionine and even the amino acid present is not released appreciably on digestion. On the other hand lysine though poorer in concentration than casein, it is released to a large extent from these leafy vegetables. Moreover, the values obtained for the vegetable powders in regeneration of serum proteins, Red Blood Corpuscles, Haemoglobin and Xanthine oxidase have been found to be comparable to those of casein. It has been found, that of the two vegetables, amaranth is superior to *Trigonella* in all the cases except digestibility. As the rate of release of lysine from the vegetable powders is of a high order, it has been suggested by the author, that these vegetables may be valuable in supplementing lysine deficient diets, such as those where cereals are used exclusively.

3. Leafy Vegetables as a Source of Minerals:

The maintenance requirement of young women were found by Blatherwick *et al* (1922)⁸⁵ to be satisfied by the calcium of lettuce, spinach or cabbage. Adolph *et al* (1932)⁸⁶ feeding rats with celery at a dietary level of 0.1 per cent calcium found 95 to 99 per cent utilization. Mallon *et al* (1933)⁸⁷ have reported that the calcium of lettuce is more

efficiently used than that of milk by young women. Lee Kao et al (1938)⁸⁸ found the utilization value of 80 per cent, when half of the calcium of the milk diet was replaced by the calcium of chinese cabbage at a dietary calcium level of 0.6 to 0.8 per cent, and have also remarked that calcium from chinese cabbage was nearly as well utilized by rats as that of milk. Basu and Gosh (1943)⁸⁹, Subramanyan et al (1949)² and Sur and Subrahmanyam (1952)⁹⁰ report from their studies, that greens such as turnip greens, amaranth, lucerne, kale, and cabbage contain large amounts of calcium and therefore they form a very good source of calcium. Basu and Gosh (1943)⁸⁹ noted that calcium from amaranth was well utilized although not to the same extent as calcium from milk. According to these authors the lesser utilisation of calcium from amaranth is probably due to the oxalic acid content of amaranth. Basu (1946)⁹¹ estimated the calcium content of samples of pumpkin leaves from different varieties, and reports that the calcium content of the leaves ranged from 240 to 300 mg. per 100 gm. of the leaves which was double the amount contained in milk, and moreover the calcium was completely utilized. Therefore, he suggests that milk could be replaced by such leaves. Sur et al (1955)⁸³ have shown that lucerne had two advantages, a) the calcium content of desiccated lucerne is twice that of milk powder and b) the utilization of calcium from lucerne is equivalent to that of milk. Mc Garrison (1956)⁵ suggests that the best way of supplying calcium both for the pregnant

women and lactating mothers is through milk and leafy vegetables.

• Leafy vegetables are also rich in iron. The presence of iron and its availability from tender amaranth leaves and stalks has been pointed out by Ranganathan (1938)⁹². Subrahmanyam (1949)² remarks that lucerne contains twice the amount of iron, in spinach.

III. EXPERIMENTAL.

This investigation was designed to find out the leafy vegetables commonly consumed in a nearby village ~~Perambalur~~ in ~~Perambalur~~ Block, ~~Perambalur~~ District, and in the urban areas of ~~Perambalur~~ City, and compare the nutrient content and availability of calcium from the mixture of wild greens called 'Kalavai Keerai', which was found to be consumed by the rural families in ~~Perambalur~~ village and amaranth found to be consumed by the urban families in ~~Perambalur~~ City. A series of experiments were ~~therefore~~ carried out in the following order.

- A. Survey of the rural and urban families to find the common leafy vegetables consumed by them.
- B. Analysis of ~~nutrients~~ and oxalic acid in kalavai keerai.
- C. Animal experiments to compare the calcium retention from amaranth and kalavai keerai.
- D. ~~Palatability~~ tests to compare the acceptability of amaranth and kalavai keerai.

The survey of the rural and urban families involved the following steps.

1. Selection of the sample.
2. Selection of the method.
3. Developing the interview schedule.
4. Collection of data.

1. Selection of the sample:

Fifty families were considered statistically adequate to constitute the samples in each category. Thus a sample of 50 families from ~~Bhambhalingan~~ village, and another sample of 50 families from three different areas of ~~Gandhinagar~~ City were selected on random basis by taking every fourth house.

2. Selection of study method:

The method selected for the collection of information regarding the kinds of greens mainly used by the selected families was the interview method, since according to Young (1956)⁹⁶ "Interviews are useful in the collection of personal data for quantitative purposes". Furthermore, as most of the villagers were illiterate, the interview was the only suitable and convenient method.

3. Developing the Interview Schedule:

Young (1956)⁹⁶ states "An interview schedule is a planned list of questions to be asked during the interview". Accordingly a list of questions was prepared to elicit information on the types, amounts, and frequency of leafy vegetables

used; the reasons for using them; the method used for cooking the leafy vegetables; and the attitudes and opinions of the homemakers regarding leafy vegetables. The interview schedule thus constructed is given in Appendix I.

4. Establishment of Rapport:

With homemakers in both the samples, rapport was established by self introduction, exchange of greetings, and explanation of the purpose of the interview. The questions were then asked one by one, and the replies of the homemakers were recorded. Table I gives the common leafy vegetables consumed by the 50 families in the urban and rural areas, indicating the frequency of purchasing range of quantities, and number of families.

TABLE I.
COMMON LEAFY VEGETABLES CONSUMED BY URBAN AND RURAL
FAMILIES.

Leafy Vegetables.	Coimbatore City (Urban)				Devangalayan (Rural)			
	Pre- quency	Quanti- ties. oss.	No. of fami- lies.	Tot- tal No.	Pre- quen- oy.	Quanti- ties. oss.	No. of fami- lies.	To- tal No.
Amaranth		1 -2	4	36	Nil	Nil	Nil	
	Daily	2 -3	8		Nil	Nil	Nil	
		4 -6	3		Nil	Nil	Nil	
	Twice	1 -2	6		Nil	Nil	Nil	
	a	2 -3	5		Nil	Nil	Nil	
	week	4 -6	2		Nil	Nil	Nil	
	Once	2 -3	4		Nil	Nil	Nil	
	a	3 -4	3		Nil	Nil	Nil	
week	4 -6	1		Nil	Nil	Nil		
Parupu- keeral	Twice	2 -3	6	10	Nil	Nil	Nil	
	a							
	week							
	Once	1 -2	4		Nil	Nil	Nil	
	a							
	week							
Arakkeeral	Once	2 -3	4	4	Nil	Nil	Nil	
	a							
	fort							
	night							
Kalayal- keeral	Nil	Nil	Nil		Daily	1 -2	3	50
					Twice	1 -2	13	
					a			
					week			
	Nil	Nil	Nil		Once	1 -1	22	
					a			
					week			
	Nil	Nil	Nil		Once a	2 -3	12	
					fort			
					night			

From the data given in Table I it is clear that amaranth was consumed by the maximum number of urban families and kalavaikeral by the largest number of rural families.

Composition of Kalavai Keeral: Kalavai keeral is a mixture of ten varieties of leafy vegetables, growing in the fields, or in the waste lands, among the weeds. The villagers, pluck these leafy vegetables and use them in mixed forms. The proportion of each leafy vegetable in the mixture depends on the availability. The names and descriptions of these leafy vegetables are discussed below with the illustrations.

1. Mairuvi (*Achyrocline aspera* Linn).
2. Mullaikeral (*Amaranthus spinosus* Linn).
3. Thoya keeral (*Digera arvensis* Persk).
4. Kovai keeral (*Coccinea Indica*).
5. Kuppekeral (*Amaranthus viridis*).
6. Herringgi (*Tribulus terrestris*).
7. Sarannai (*Portulaca portulacastrum*).
8. Sivenar Vembu (*Justicia tranquebariensis* L.F.).
9. Palla keeral (*Pergularia orbensa*).
10. Pumpkin leaves (*Cucurbita pepo*).

1. Mairuvi: (Figure 1)^{p.39} This plant is erect, sparingly branched, perennial, with the height of one to three feet. The stem is round, straggled and hairy. The leaves are simple and are exstipulate and shortly stalked. The inflorescence is an elongate terminal spike attaining a length of even two feet.

The fruits are single seeded and easily stick on to the skins of animals or the clothing of human beings and get dispersed. The plant is highly medicinal, and used for treatment of various diseases, especially for piles.

2. Mullukuturai: (Figure 2)^{p.40}. Mullukuturai is an erect, or decumbent annual herb, flowers are small sessile in axillary clusters. This plant is very commonly found in the neglected waste places and occasionally in fields and gardens. As the specific name indicates it is easily made out from the thorns found in the leafy axils and at the base of inflorescence. The stem is round, and stout, and the leaves are simple, alternate, exstipulate with long petioles. It is an antidote for snake bite.

3. Thoya keerai: (Figure 3)^{p.41}. Thoya keerai is one of the most common weeds found in all kinds of soils. It thrives best in most loamy soils of the cultivated fields. The stem is round, striate, glabrous, or very sparsely hairy. The leaves are simple and alternate. The flowers are pink, sessile, occurring in groups of three and are arranged in lax axillary spikes.

4. Korai keerai: (Figure 4)^{p.42}. This plant is a Prostrate or climbing herb with ~~branches~~ and a thick tap root. The branches are glabrous, grooved, and have simple tendrils. The leaves are simple, alternate and ex-stipulate. They are often glabrous, but are ~~sensitively~~ tough with papillae. These leaves are medicinal and used for fevers, and the tap root is useful in the treatment of diabetes.

5. Muppakuralai: (Figure 5) p.43. Muppakuralai is an erect, glabrous, and branched plant. The leaves are single, alternate, exstipulate with long petioles. The flowers are arranged in terminal or axillary elongate panicles. The leaves are eaten by the poor people.

6. Merringgi: (Figure 6) p.44. Merringgi is a prostrate herb, with pinnate leaves and yellow solitary flowers. It is a branched spreading herb with dense hairy stems. The entire plant is a cooling tonic, diuretic, and used in urinary disorders.

7. Saravali: (Figure 7) p.45. This plant is a most conspicuous and aggressive weed of cultivation in South India. It thrives well in both dry and garden lands. It is a succulent herb, with round and striate stem. Branches arise from every node. Leaves are fleshy in opposite sides on equal pairs, and are round, ovate, glabrous, green above and pale and glistening below. This plant is good for rheumatism. It is an antidote for alcoholic poison and should not be taken with gingelley oil.

8. Sivenar Vembu: (Figure 8) p.46. Sivenar vembu is an erect and non-bushy ^{plant.} The leaves are small round, and are attached to the nodes. At each node there is a cluster of leaves with small seeds. The flowers are pink and white and are very small in size. This is also eaten ~~by the poor people.~~ by the poor people.

9. Kalla Kanni: (Figure 9) p.47. This plant is a prostrate, or climbing plant with branches and tendrils. The leaves are simple and alternate, and the surface of the leaves are very

smooth and slightly velvety.

10. Pumpkin leaves: (Figure 10)^{p48}. This plant is an under shrub, climbing by means of tendrils. This plant belongs to gourd's family. The leaves are simple, alternate, and exstipulate. This widely grows in tropical warm areas.



Vast differences are found between the urban and rural families in the methods of cooking greens and time taken for cooking them as shown in Table II.

TABLE II .
METHODS USED FOR COOKING LEAFY VEGETABLES
BY URBAN AND RURAL FAMILIES.

Coimbatore City (Urban)			Devlanpattanam (Rural)		
Method of cooking.	Time. mts.	No. of families.	Method of cooking.	Time. mts.	No. of families.
<p>1. <u>Korial:</u></p> <p>The greens are washed, cut and cooked in just enough water along with onions, chillies and salt and seasoned.</p>	6	50	<p>1. <u>Korial:</u></p> <p>The greens are cut, washed and cooked in excess water and seasoned after discarding the excess cooking water.</p>	8	50
<p>2. <u>Maseal:</u></p> <p>The greens are washed, cut, and cooked in just enough water along with onions, chillies and salt and mashed and seasoned.</p>	8	42	<p>2. <u>Maseal:</u></p> <p>The greens are cut, washed and cooked in excess water, and mashed with salt after discarding the excess water, and seasoned.</p>	10	50
<p>3. <u>Keerali - Dhal Kootu:</u></p> <p>The red gram dhal is put and mashed. Greens are washed, cut and cooked separately with onions, chillies and salt. The cooked greens are then mixed with the mashed dhal and seasoned.</p>	10	26	<p>3. <u>Keerali - Dhal Kootu:</u></p> <p>The red gram dhal is cooked and mashed. Greens are cut, and washed and cooked with onions, chillies and salt in excess water. The cooked greens are mashed after discarding the excess cooking water and mixed with the mashed dhal and seasoned.</p>	13	20

From Table II it is clear that the maximum number of families in both the urban and rural areas prefer "Porial" and "Maseal". Hence these two preparations were selected by the investigator to compare the palatability of amaranth and kalavai keerai.

Information regarding the popular attitudes and notions about greens was collected and the reasons given by the urban homemakers are tabulated in Table III.

TABLE III.
REASONS GIVEN BY THE URBAN HOMEMAKERS FOR INCLUDING LEAFY VEGETABLES IN THE DIETARIES.

Reasons.	No. of families.	Per-centage
1. Avoids constipation	50	100
2. Easy to cook	49	98
3. Nutritious	48	96
4. Heals ulcers in mouth and stomach	46	92
5. Very tasty	40	80
6. Rich in iron	31	62
7. Adds variety to the diet	22	44
8. Very attractive	16	32
9. Purifies blood	10	20
10. Helps in hair growth	4	8
11. Good for nerves	3	6
12. Very cheap	2	4
13. Improves complexion	1	2

From Table III it is evident that many homemakers have some knowledge regarding the nutritive value of greens. It is surprising that only a few homemakers (4 per cent) accept the

fact that greens are cheap. So it is evident that the high nutritive value of greens has popularized its use rather than its low cost. In the case of rural inhabitants, without any exception every one is of the opinion that leafy vegetables are very nutritious and good for health.

Thus it is clear, that there are wide differences between the urban and rural people with regard to frequency of including greens in the dietaries, and the method of cooking greens. The urban people do not discard the cooking water and fifteen families use greens regularly; where as in rural areas all the people discard the cooking water, and the time taken for cooking greens by the rural folk is comparatively greater than those of urban people as shown in Table II.

This survey revealed that the widely used leafy vegetables by the urban and the rural families were amaranth and kalavai keerai respectively. Knowledge regarding the nutritive value and supplementary effect of amaranth on rats fed on poor rice diet is available, but as there is no information on the nutritive and growth promoting value of kalavaikeerai, it was selected as the subject of study for this investigation, to be compared with amaranth. For experimental purposes equal quantities by weight of all the ten varieties of wild greens were taken to constitute the mixture, 'Kalavaikeerai'.

B. Analysis of Nutrients and Oxalic Acid in Kalavaikeerai:

Only kalavaikeerai was analysed for the following.

nutrients namely moisture, calcium, iron, vitamin C and Ca carotene, since the nutritive value of amaranth is already known (Aykroyd et al 1962)¹¹.

Edible portions of the ten different leafy vegetables in kalavai keerai mixture were cleaned and taken in equal quantities by weight and dried at 65° C in hot air oven, for two days. The dried leaves were powdered with a mortar and pestle and used for the determination of calcium, phosphorus, iron, protein and carotene. For the determination of vitamin C and moisture 10 gm. of fresh leaves of each of the ten varieties were mixed and used. The methods adopted for the determinations of all the nutrients except vitamin C were those of Jacobs (1958)⁹⁴. Calcium was precipitated as the oxalate and the amount of calcium determined by means of permanganate titration. Phosphorus was estimated by titrimetric method, in which the phosphorus in a test material is converted to phosphomolybdate dissolved in standard alkali, and the excess alkali is titrated against standard acid. Iron and carotene were determined colorimetrically using Klett Summerson calorimeter. Vitamin C was estimated by the 2:6 dichlorophenol indophenol Visual Titration method, as described by the Association of Vitamin Chemists (1947)⁹⁵. Since the oxalic acid contents of leafy vegetables affect their utilisation in the body, (Giri (1940)³⁸, Johnston et al (1952)³⁹ and Subrahmanyam et al (1961)³⁴, kalavaikeerai was also analysed for its oxalic acid content following the method described by Majumdar (1938)⁹⁶. "10 to 20 gm. of the fresh test substance

were ground with glass powder in a mortar and the whole mate. was transferred to a beaker. It was then boiled with 15 per cent hydrochloric acid, filtered over a Buchner funnel and thoroughly washed. The filtrate was made alkaline with ammonia, reacidified with acetic acid and finally precipitated hot with calcium chloride solution. This was filtered and the residue boiled with acetic acid and again filtered, after which it was thoroughly washed with water to make it free from chloride. The filter paper was ignited and ashed in the electric furnace. The calcium oxide was next dissolved in a little hydrochloric acid, made alkaline with ammonia, and precipitated with ammonium oxalate solution. The calcium oxalate thus formed was filtered and washed free from the soluble oxalate and titrated with standard potassium permanganate solution.

G. Animal experiments to Compare Calcium Retention from Amaranth and Kalavaikcerai:

In order to compare the retention of calcium from kalavaikcerai with amaranth, albino rats were chosen as the experimental animals, because as referred by Farris (1959)⁹⁷, rats are widely used in the laboratories, because of their low cost, small space requirement, tractability, omnivorous dietary, short time span of generations, large litters, and they can be readily standardized.

The steps taken for this investigation are:-

1. Selection and preparation of the basal diet,
2. Preparation of experimental diets,
3. Selection of equipment,
4. Selection of the rats,
5. Recording the food consumption of rats,
6. Recording other observations on rats,
7. Recording weights of rats,
8. Conducting calcium balance studies, and
9. Determining the calcium content of femur and tibia.

1. Selection and Preparation of Basal Diet:

The "Poor Rice Diet" used by Mason and Thompson (1945)¹⁴ was selected as the basal diet for this study, since it is typical of the South Indian rice diets consumed by a majority of the people. The composition of the Poor Rice Diet is given in Table IV.

TABLE IV.
PERCENTAGE COMPOSITION OF THE BASAL DIET.

Ingredients.	Amount in grams.
Par boiled rice (milled) flour	89.70
Redgram dhal flour	3.00
Brinjal	4.30
Raw plantain	2.10
Gingelly oil	0.43
Mutton	0.26
Coconut	0.21
Distilled water	1/2 cup.

From the ingredients given in Table IV the basal diet was prepared as follows:-

The vegetables, mutton and coconut were minced and mixed thoroughly with the other ingredients, steamed for 25 minutes in an iddly steamer, and made into balls each weighing about 50 gm. The ratio of the weight of uncooked to cooked diet was 1: 1.7.

2. Preparation of Experimental Diets:

The two experimental diets used in this study were:

1. Basal + dehydrated amaranth powder.
2. Basal + dehydrated kalavaikcerai powder.

The amounts required to meet at inessential level, the optimum calcium requirement for the rat which is 50 mg. of calcium per day per rat according to Farris and Griffith (1959)⁹⁷ was 1.5 gm. of dehydrated amaranth powder and 2.6 gm. of dehydrated kalavaikeeral powder.

The edible portions of each of the ten varieties in the kalavaikeeral mixture were picked, and equal weights of 50 gm. mixed together, washed in distilled water, and dried at 65° C in an electric hot air oven. The dried sample was powdered, sieved through a 30 mesh sieve and stored in a dark blue bottle with a tight bakelite lid. The same procedure was adopted for preparing the dehydrated amaranth powder which was stored in a dark brown bottle, in order to distinguish it from the other.

The required amounts of dehydrated amaranth and kalavaikeeral powders, that is 1.5 gm. and 2.6 gm. respectively, were each mixed with one teaspoonful of water and steamed separately for 30 seconds. The steamed leafy powders were then mixed with 50 gm. of the cooked basal diet and fed to rats.

3. Selection of Equipment:

The equipment used for the experiments were:-

a) ordinary rat cages, b) metabolic cages, c) ohaus weighing balance with animal box, and d) the apparatus for calcium determinations.

a. Ordinary Rat Cages:

Rectangular cages made of aluminium 12" long, 8" wide and 9" high were used in this study. The bottom of the cages consisted of raised wire mesh, under which were placed sliding trays, which collected the spilt food and the excreta. Each cage had a card plate in which a card, as shown in appendix II to note down the particulars such as the cage number, the diet given, the daily food intake, the weekly weight of the rats and observation on appearance and deficiency symptoms could be inserted. The bottom trays for collecting the spilt food and the excreta could be easily removed and washed daily. The cages were washed with soap and phenyl twice a week and sun dried.

For feeding the rats, enamel plates of 5" diameter were used in order to minimise spillage. For supplying drinking water, bottles with metallic tubes at their mouths were used. They were fixed on the door of the cages in an inverted position, as shown in Figure 11, so that the rats had access to these tubes, through which they could drink water. Distilled water was the source of drinking water.

b. Metabolic Cages:

The metabolic cages were cylinder shaped with a wire mesh bottom. A plain aluminium funnel was attached to the bottom of these cages as shown in Figure 12, to collect the drippings of urine. Glass bottles for collecting the urine were placed under



Fig. 11 Ordinary rat cage

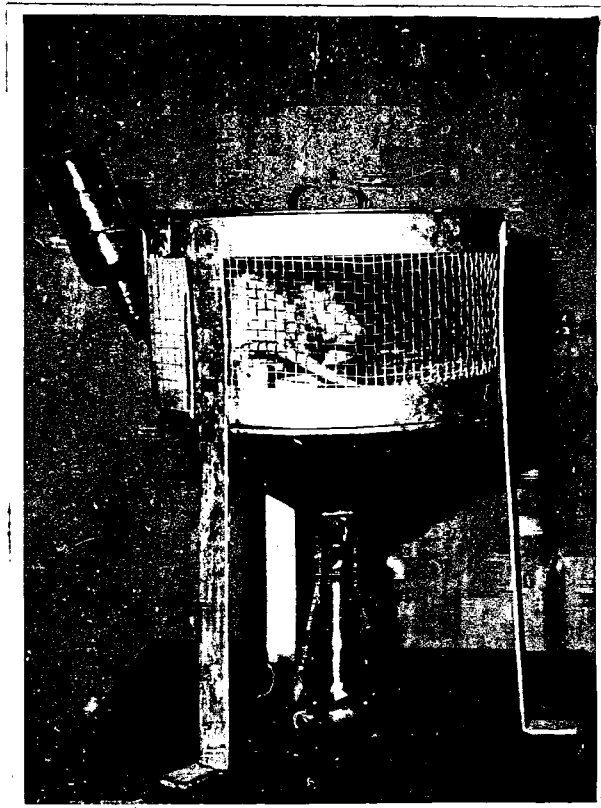


Fig. 12 Metabolic Cage

the funnels and the nozzles of the funnels were inserted in the neck of the bottles. Glass wool was placed in the nozzles of the funnels to prevent pieces of spilt food and faeces, falling into the bottles. As in the case of the ordinary cages, drinking water bottles were fixed, and the diets served on enamel plates.

3. Glass Weighing Balance with animal box:

An ohaus weighing balance with an animal box as shown in figure 13 was used for weighing both the diets and the rats. It was graduated in grams from 0.1 gm. to 500 gm.

4. Apparatus needed for calcium determination:

The apparatus needed for calcium determinations were, glass beakers, funnels, whatman No. 44 filter paper, wash bottles, porcelain files, glass rods, funnel stands, Bunsen burners, silica dishes, pipettes, and micro burette.

4. Selection of the Rats:

Twelve, 28 days old male albino rats of the Wistar strain were used for this study. They were divided into three groups of four rats, each group maintaining the same average weight (48.5 to 549 gm.)

One group was used for the basal diet and constituted the control group. The remaining two groups were supplemented with amaranth and ~~Maintenational~~ powders respectively, and formed

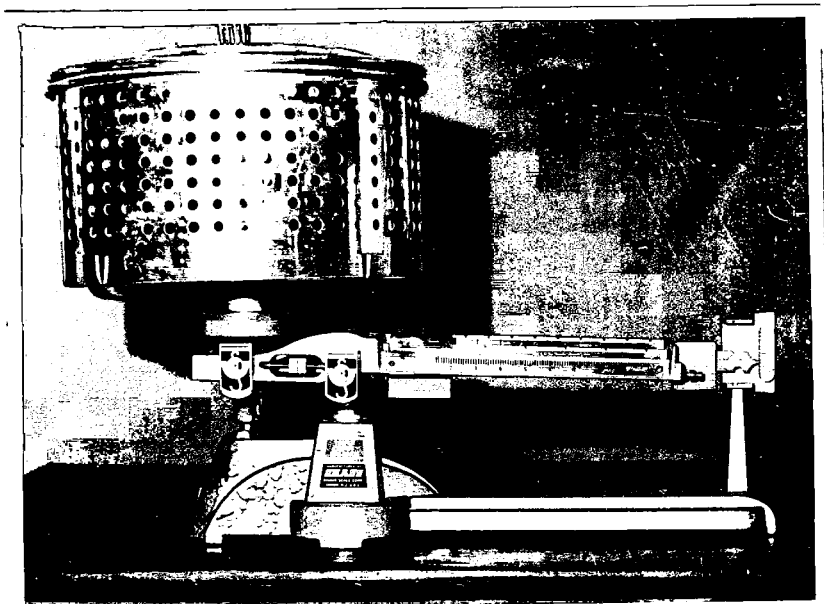


Fig. 13 Ohaus rat weighing balance

the experimental groups.

5. Recording the Food Consumption of Rats:

The amount of food given daily to each rat was so adjusted, that about 10 to 15 gm. of the food would be left over, to ensure that the rats had enough to eat, since the feeding was ~~ad libitum~~. The records of daily food consumption were maintained by noting the weight of the food given and the weight of the food left over to the nearest one tenth of a gm. In order to determine the losses in weight due to ~~evaporation~~ of moisture from the diets, an aliquot of the diet was placed ~~simultaneously~~ in an empty cage in the laboratory, and weighed again the next morning. From this procedure, the average moisture loss was found to be three per cent of the fresh weight. This correction was made while calculating the actual food intake by the rats.

6. Recording other observations on rats:

The general appearance of the rats, their activities, eyes, skin, tail coat, and whiskers were daily observed. Any symptoms of deficiencies or abnormalities were recorded on the cards.

7. Recording weights of rats:

The four rats in each group were weighed on the first day of the experiment and kept in individual cages, each of which

carried the cards to note the actual data of weaning of the rat, the cage number, the diet given, the daily food intake and the weekly weights of each rat. The rats were weighed every week to the nearest one tenth of a gm. and the weights recorded with dates, for eight weeks.

8. Conducting Calcium Balance Studies:

The following experiments were carried out for the Calcium Balance studies:-

- a) Calcium estimation of faeces.
- b) Calcium estimation of urine.
- c) Calcium estimation of basal and experimental diets.

During the second and eighth weeks of the experiment, all the rats were kept in the metabolic cages for the whole week. The collection of faeces and urine was made during the last four days of the respective weeks for estimating the calcium contents.

The reasons for conducting the calcium balance study during the second week of the period was to allow one week for the rats to get adjusted to the new surrounding and foods, and another calcium balance study at the end of the experimental period (eight weeks) was conducted to find out whether there was any difference in calcium retention due to age.

a) Calcium estimation of faeces:

The faeces were collected from the screen bottom of

cages every day carefully, removing the adhering food particles, stored in beakers with sufficient methanol to cover the faeces and kept in the refrigerator (Sugamony 1959)⁷⁵. All the faeces collected during the period of four days were stored in the same beaker. The method followed for the calcium analysis in the faeces was as follows:

The faeces were dried at 65° C in an hot air oven until constant weights were reached. The dried faeces were ^{ashed,} dissolved in 10 to 15 ml. of concentrated hydrochloric acid and made up to 50 ml. with distilled water. Twenty ml. aliquots of the ash solution were taken for the estimation of calcium using the oxalate permanganate titration method, to which 30 ml. of saturated ammonium oxalate solution and 30 ml. of ammonia were added and boiled for one minute. While the solution was still hot, two drops of glacial acetic acid were added, and kept aside covered with a watch glass over night, to facilitate precipitation of calcium oxalate. The calcium oxalate precipitate was filtered through quantitative Whatman No. 44 filter paper, and the precipitates washed with hot distilled water, filling the funnel about two thirds full, and allowing it to drain completely each time. The washings were tested for chlorides by collecting 5 ml. in a test tube, adding a drop of 5 per cent silver nitrate solution. When the washings were free from chlorides, a hole was made in the centre of the filter paper and the precipitates were washed into a beaker with 30 ml. of 6 N Sulphuric acid, and heated to 60° to 70° C and titrated against

standard potassium permanganate (0.1042 N).

b) Collection and analysis of urine:

The urine for calcium analysis was collected in bottles containing 10 ml. of the preservative, that is equal quantities of toluene and concentrated hydrochloric acid, placed beneath each funnel during the four day collection period. At the end of the collection period, the funnels were washed with distilled water, and the washings were added to the urine in the bottles,

McCrudden's (1911)⁹⁸ method was used for the analysis of calcium in the urine. The urine thus collected was made up to 200 ml. with distilled water, and filtered. When it was faintly acidic to litmus paper, ten ml. of concentrated hydrochloric acid, ten ml. of 2.5 per cent oxalic acid and eight ml. of 20 per cent sodium acetate were added, and the mixture allowed to stand over night at room temperature to maximise the precipitation of calcium oxalate. The next day the calcium oxalate precipitate was filtered using quantitative Whatman No. 44 filter paper and washed free from chlorides with 0.5 per cent. ammonium oxalate solution. When the precipitate was free from chlorides, a hole was made in the filter paper and the calcium oxalate washed into a clear flask with distilled water, and the volume made up to 50 ml. Ten ml. of concentrated sulphuric acid were added and titrated with standard potassium permanganate until a pink colour which lasted for at least a minute resulted.

8) Calcium estimation of Basal and Experimental Diets:

During the collection period an equal portion of the diets served to the four rats in each group, that is a fifth portion was kept in a beaker and placed in the refrigerator. After the collection period of four days, the diet samples were homogenised, and aliquots ashed to estimate the calcium content.

9. Estimating the Calcium Content of Femur and Tibia:

The femur and tibia were removed from the rats, according to the method described by Calverley *et al* (1949)⁶⁷ and analysed for their calcium content. At the end of the eighth week, the rats were chloroformed and the right femur and tibia ^{were} removed carefully and soaked in ether to dissolve and remove the adhering fat particles. After soaking in ether for two to three hours, the bones were washed again with ether and dried to constant weights at room temperature. The dried bones were then ashed and the calcium content determined as described by Jacob (1958)⁹⁴.

D. Palatability Tests to Compare ^{the} Acceptability of Amaranth and Kalavaikeral:

Cooking experiments were conducted to judge the palatability and acceptability of various preparations of kalavaikeral by a panel of judges and compared with those of amaranth.

These involved:-

1. Selection and Identification of leafy vegetable preparations.
2. Selection of the tasting panel.
3. Developing the Score card.

1. Selection and Identification of Leafy Vegetable Preparations:

As was mentioned in Table II on page 49, 'Porial' and 'Masal' were found to be the most common leafy vegetable preparations. These two preparations were therefore selected for the palatability tests.

2. Selection of the Tasting Panel:

Sweetman *et al* (1954)⁹⁹ state that the most satisfactory way to test foods for texture, flavour and palatability, is to serve the samples to expert human judges and compare their verdicts.

In order to select the tasting panel the Triangle Test method described by Lowe (1955)¹⁰⁰ was adopted, in which the test unit consists of three food samples, two of which are aliquots of the same sample. All the three samples are coded, so that they are known only to the person who is administering the test, and not to the persons who judge. The members of the panel are requested to pick the "different" sample, and indicate the reasons for their decision. After three consecutive tests, those who had been consistent in pointing out the "different" sample correctly, are selected to be the members of

the tasting panel. In addition to their ~~sensitivity~~ to the ~~difference~~ in taste and flavour as indicated by Lowe (1955)¹⁰⁰, the judges should be healthy and free from cold and ~~infections~~ in the sense organs.

With the above points in view, the Triangle Test was administered to six members, all of whom were Home Science graduates of Sri ~~Avinashilingam~~ Home Science graduates of Sri ~~Avinashilingam~~ Home Science College, ~~Chennai~~. They ranged in age from 20 to 25 years, and were in perfect health ~~condition~~, and had a high degree of personal integrity and ~~willingness~~ to co-operate in judging the products.

Two equal parts of the same samples of amaranth were cooked ~~separately~~ using soft water, and hard water ~~respectively~~. To constitute the unit for the Triangle test, two ~~portions~~ from one part and one from the other were presented to the judges.

The Triangle Test was ~~conducted~~ three times. The cooked products were served on white porcelain plates in identical ~~quantities~~ which were ~~sufficient~~ for the purpose of testing. The sample score card given to the judges to pick the "different sample" is given below:-

TABLE V.

SCORE CARD FOR USE IN TRIANGLE TEST.

Date:

No:

Sample:

Different sample	How is it different?

Signature of the Judge.

Instruction: Among the three samples given two are identical and one is different. Kindly mark which is the different sample and how it is different. After tasting each sample please drink little water and pause a while to remove the taste of that sample. Then taste the next sample.

All the six graduates were found to be consistent and therefore selected to constitute the tasting panel.

2. Developing the Score Card:

A score card as shown in Appendix III was developed in order to help the six judges to carry out the objective rating of the cooked preparations of the leafy vegetables. The scores were accompanied by descriptive terms in a graded manner. The criteria listed in the score card were mainly based on the qualities, usually looked for in leafy vegetable preparations

such as colour, texture, taste, and other prominent characteristics like the slimness of the products. Accordingly three descriptions for each of the qualities were tabulated, the descriptive terms ranging from the superior to the inferior.

IV RESULTS AND DISCUSSION.

The results of the experiments conducted are discussed below from three angles. They are as follows—

A. Analysis of Kalvai Keerai.

B. Availability of Calcium

1. Growth Study

2. Calcium balance study

a) Digestibility

b) Calcium retention

3. Calcium content of femur and tibia.

C. Palatability tests.

A. ~~Analysis of Kalvai Keerai~~—analysis of Kalvai

Keerai was carried out and the results are given below in table VI.

TABLE VI.
ANALYSIS OF KALAVAI KEERAI.
(Values per 100 gm. on wet basis)

Name of Leafy Vegetables	Moisture gm.	Protein gm.	Calcium gm.	Phos- phorous gm.	Iron gm. mg %	Caro- tone in terms of Vit. A. I.U.	Vit. C gm.
*Kalvai Keerai	75.5	1.8	0.50	0.10	126.8	7,230	95
* Anaranth	85.8	4.9	0.50	0.10	21.4	2,500 to 11,000	173

* Values taken from ~~Report~~ ~~Publication~~ ~~Series~~ (Agriculture, 1955)

From the results it is found that Kalavai Keerai is a rich source of minerals and carotene, and when compared with amaranth it has a high content of phosphorus, while it is poor in Vitamin C. content. The low Vitamin C content may be attributed to the fact that when the Kalavai Keerai was analysed for its vitamin C content, it was not in a very fresh condition, because the investigator had to go 15 miles away from her residence to collect the greens, and the collection of the greens was done in the morning and the estimations could be carried out only in the evening. Part of the vitamin C in the greens might have been lost during this time. The same explanation may be given for moisture content also. Since the calcium content of amaranth and Kalavai Keerai are more or less the same, the investigator was interested to compare the availability of calcium from Kalavai Keerai with that of amaranth. Hence biological studies are discussed below.

B. Availability of Calcium.

The criteria used for judging the availability of Calcium were (1) growth study (2) Calcium balance study and (3) Estimation of Calcium content of femur and tibia.

Table VII. gives the data regarding the average weights and the analysis of the mean differences of the weights gained by the rats during a period of eight weeks on basal and experimental diets, which were calculated from the data given in Appendix IV.

TABLE VII.
AVERAGE GAIN IN WEIGHT BY ALBINO RATS FED ON BASAL AND
EXPERIMENTAL DIETS DURING EIGHT WEEKS.

Diets	Average initial weight gm.	Average final weight gm.	Weight gained gm.
Basal diet	49.0	85.2	36.2
Basal + Amaranth	48.4	101.7	53.3
Basal + Kalavai keerai	49.0	106.7	55.7

ANALYSIS OF MEAN DIFFERENCES OF WEIGHTS

Comparison of value.	Mean Dif. gm.	* S.E. gm.	** 't' Value.	Level of significance.
Basal against basal + Amaranth	17.1	6.9	2.48	P < 0.05
Basal against basal + Kalavai Keerai	19.5	7.2	2.70	P < 0.05
Basal + Kalavai Keerai against basal + Amaranth	2.4	7.3	0.32	P > 0.05

* S.E. (Standard error).

$$S.E. = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n_1} + \sum y^2 - \frac{(\sum y)^2}{n_2}}{n_1 + n_2 - 1}}$$

n_1 = Number of rats

n_2 = Number of rats

** 't' = $\frac{\text{Mean difference}}{\text{Standard Error of the mean difference}}$

According to Walker (1953), 't' value measures the significance of the differences between means of the groups.

As seen from Table VII there is a significant difference between basal and experimental groups regarding growth, but within the experimental diets the difference is not significant. Absence of significant difference between the two experimental diets denotes that as a supplement to 'poor rice diet' both these greens have equal value, regarding growth promotion.

Using the mean values given in Appendix IV, the growth of rats in each group is graphically represented in Figure 14

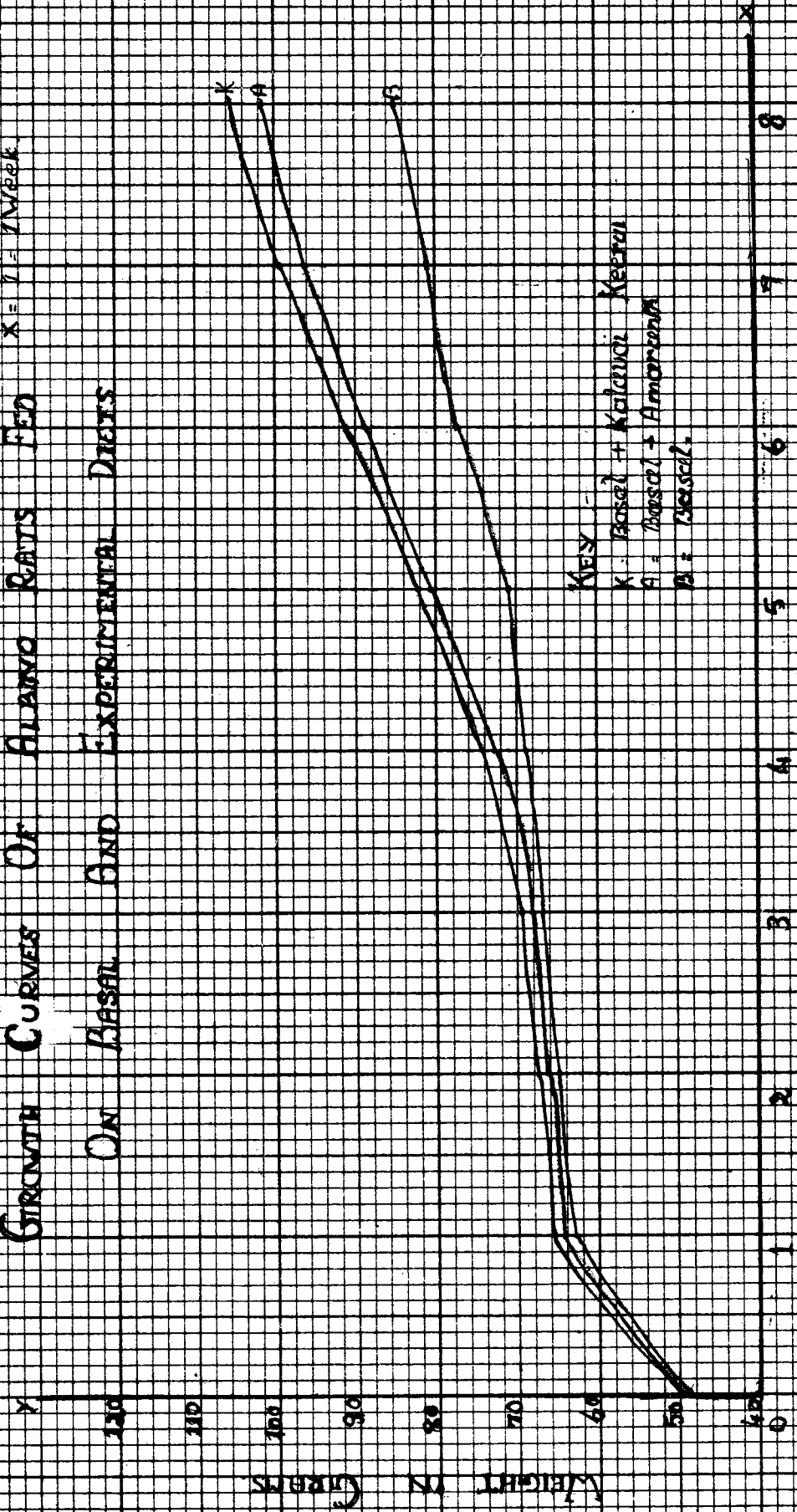
As shown in Figure 14 ^{p. 72 There is} regular increase in weights of the experimental groups. A steep increase is seen in the growth curves of rats in all the three groups, in the first week, after which the growth rates are gradual and almost parallel. By the end of the third week the differences are distinctly shown, and the growth levels of the experimental groups are much higher than the basal group, which proves that both the leafy vegetables enhance the growth of rats fed on 'poor rice diet'. On comparing the growth curves, the curve of rats fed on 'basal plus Kalavai Kerali' is at the highest level^E, proving the superiority of Kalavai Kerali over amaranth for growth.

Physical appearance and activities of the rats

Physical appearance of the rats in all the three groups was observed by the investigator throughout the experimental period, and any external deficiency symptoms present in the rats were recorded. The photographs of the rats in each group are shown in Figures 15, 16 and 17 on pages 73, 74 & 75

STRENGTH CURVES OF ALBINO RATS FED ON BASAL AND EXPERIMENTAL DIETS

Y = 1" = 20 grams
X = 1" = 1 WEEK



KEY -
 K - Basal + Kalanchoe Keenan
 A - Basal + Amaranth
 B - Basal

FIGURE 11



Fig. 15 Basal diet



Fig. 16 Basal + Amaranth



Fig. 17. Basal + Kalavai keerai

Regarding the deficiency symptoms, one of the poor rice diet rats showed signs of alopecia and developed a patch of 2" diameter on the left side, a little above the hind leg (Figure 15.) This rat was further observed for its physical activity, and it was noted to be lethargic and very timid, but its food intake was quite normal. In the case of other rats, when food was served, they would run eagerly to catch the plates, whereas this particular rat would try to hide itself in one corner of the cage. In general, much difference was not seen in the appearance of the rats in all the three groups, except in the case of the particular 'poor rice diet' rat which had developed alopecia.

2. Calcium balance study:-

The data of Calcium balance study were analysed from the angle of (a) Digestibility (b) Calcium retention.

a) Digestibility:- The extent of the digestibility of these two leafy vegetables was calculated by adopting the formula given by Bender (1958)

$$\text{True digestibility} = 100 \times \frac{\text{Food Calcium} - \text{Faecal Calcium}}{\text{Food Calcium}}$$

From the data given in Appendix V the average digestibility coefficient and the analysis of mean differences were calculated and given in Table VIII.

TABLE VIII.

DIGESTIBILITY CO-EFFICIENT OF AMARANTH AND KALAVAI KEERAI DURING THE SECOND AND EIGHTH WEEK.

Diets	2nd week		8th week	
	Diets	Digestibility co-efficient	Diets	Digestibility co-efficient
Basal + Ama-ranth		50.5%		50.7%
Basal + Kalavai Keerai		57.5%		57.7%

ANALYSIS OF MEAN DIFFERENCE OF THE DIGESTIBILITY CO-EFFICIENT OF AMARANTH AND KALAVAI KEERAI.

Comparison of diets	2nd week				8th week			
	Mean Diff-erence	S.E.	't' Value	Level of sig-nifica-nce	Mean diff-erence	S.E.	't' Value	Level of signifi-cance
Basal + Amaranth against Basal + Kalavai Keerai	7.0	2.6	2.6	P < .05	7.0	1.8	3.9	P < .01

From the contents of Table VIII it is evident that there is significant difference in digestibility co-efficient between kalavai keerai and amaranth, in which kalavai Keerai stands superior to amaranth. In the 8th week of the experiment difference in the digestibility co-efficient of

the two leafy vegetable is significant at 1 per cent level, whereas in the 2nd week it is significant only at 5 per cent level.

b) Calcium retention:- After finding the digestibility coefficients of these two leafy vegetables, the average per cent Calcium retention and the analysis of the mean differences were calculated and are presented in Table II.

TABLE IX.

AVERAGE PERCENTAGE RETENTION OF CALCIUM BY ALBINO RATS
DURING THE SECOND AND EIGHTH WEEK.

2nd Week.

Diets	Avg. dietary Ca.	Avg. faecal Ca.	Avg. urinary Ca.	Avg total excretion	Bala nce	Avg percent- age reten- tion
Basal diet	1.8	2.0	1.8	3.8	-2.0	--
Basal + Ama- ranth	27.8	13.5	6.0	19.5	8.3	28.5
Basal + Kala- vai Keerai.	26.7	11.0	4.0	15.0	11.7	43.9

8th Week.

Basal diet	2.5	2.1	2.1	4.2	-1.7	--
Basal + Ama- ranth	31.5	15.5	8.0	23.5	8.0	25.6
Basal + Kala- vai Keerai.	31.7	13.2	7.5	20.7	11.0	34.5

ANALYSIS OF MEAN DIFFERENCE OF THE RETENTION OF CALCIUM FROM
AMARANTH AND KALAVAI KEERAI.

Comparison of diets	<u>2nd week</u>			<u>8th Week</u>				
	Mean differ ence	S.E.	't' Value	Level of Signi- ficance	Mean differ ence	S.E.	't' Value	Level of Si- gnifi- cance
Basal + am aranth ag- ainst Basal. Kalavai Keerai	15.40	3.3	4.6	P < .01	8.9	3.4	2.6	P < .05

From Table IX it is clear that the maximum calcium retention is in the case of 'Basal + Kalavai Keerai'. The difference between the two experimental diets is highly significant in the second week, while the difference is just significant at the eighth week when compared with the second week which can be attributed to the differences in age. As McCay (1956)¹⁰³ reports, there is slight decline in the calcium retention value, as age advances.

The increased retention of calcium from 'Kalavai Keerai' might be due to the increased digestibility of these greens, and the decreased oxalic acid content of 'Kalavai Keerai', since 'Kalavai Keerai' and amaranth contained 0-5 gm., and 0.7 gm. of oxalic acid per 100 gm., of leafy vegetables respectively, according to an estimation carried out by the investigator adopting K Majumdar's (1938)⁹⁹ method. As Brine (1955)³² states that oxalic acid reacts with soluble calcium and renders it unavailable, this is probably another reason for the increased retention of calcium in the case of Kalavai Keerai.

It is also evident from Table IX that the rats on basal diet were in negative calcium balance due to the intake of calcium being so low as 10 mg. per 100 gm., of the food. As the rats could not meet their daily calcium requirement the body calcium was depleted.

3. Calcium content of Bone and Tissue-

Since the retained calcium is being stored in bone cells (Davidson) (1959)¹² the investigator estimated the extent of

storage of the retained calcium in the bones. From the data given in Appendix VI, the average initial and final calcium content of bones (Femur and Tibia) and the analysis of mean differences were calculated and presented in Table X.

TABLE X.
AVERAGE CALCIUM CONTENT OF FEMUR AND TIBIA

Diets.	Average initial Ca. content. mg.	Average final Ca., content mg.	Net Ca. content mg.
Basal diet	18.2	20.2	2.0
Basal + Amaranth	18.2	42.6	24.4
Basal + Kalavai Keerai	18.2	49.4	31.2

ANALYSIS OF MEAN DIFFERENCES OF CALCIUM CONTENT OF FEMUR
AND TIBIA

Comparison of diets	Mean differ- ence	S.E.	't' value	Level of significance
Basal against basal+ amaranth	22.4	0.11	203.0	P. < .01
Basal against basal+ Kalavai Keerai	29.2	1.30	22.6	P. < .01
Basal + amaranth against Basal + kalavai Keerai	6.8	0.20	34.0	P. < .01

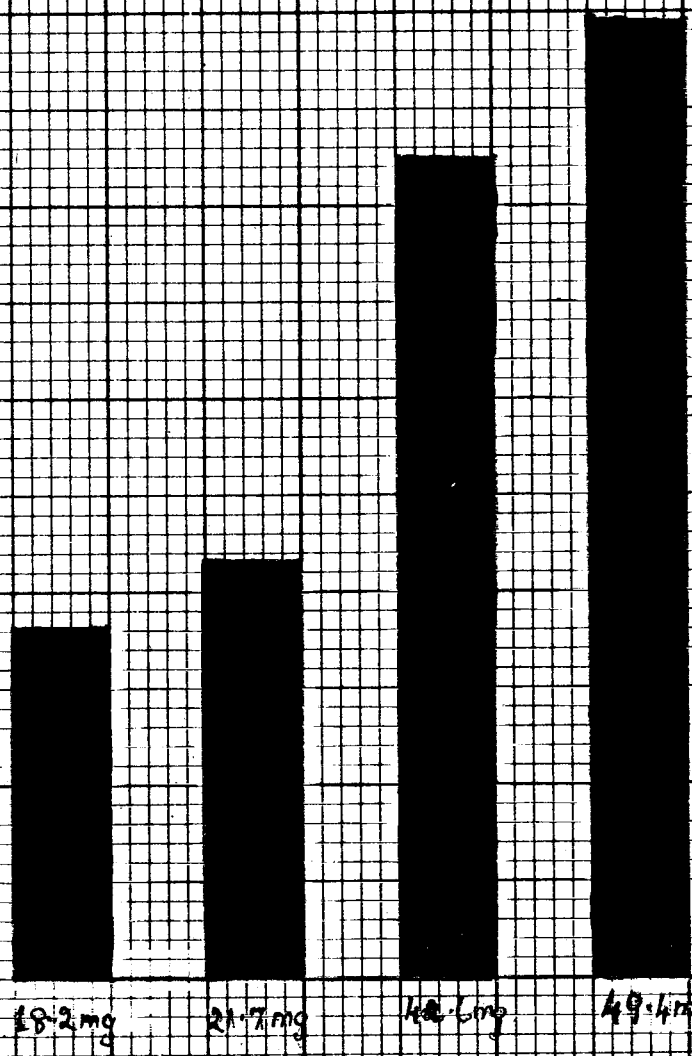
As can be seen from Table X, the differences in the calcium content of femur and tibia are highly significant. The net calcium content of the respective bones in the case of rats fed on experimental diets is eight to ten times greater than that of the basal group, the maximum content being in the case of rats fed on 'basal + Kalavai keerai'. This shows that the availability of calcium from Kalavai Keerai for calcification of bones is definitely greater than ~~amaranth~~. This same point is further emphasized through Figure 18 on page 82a

The figure shows that the femur and tibia of rats fed on 'basal + Kalavai Keerai', have the maximum calcium content while 'basal + ~~amaranth~~' and 'basal' come next in line in the descending order.

G. Palatability Studies:-

As a result of the three types of studies conducted, namely growth, calcium balance, and calcium in bone 'Kalavai Keerai' proved to be superior to ~~amaranth~~. From a survey conducted in ~~Calicut~~ / City in three different areas, it was found that the urban people have not realized the importance of such valuable leafy vegetable mixtures, so the investigator carried out a palatability test as mentioned by Lowe (1955)¹⁰³ to ascertain how far the new food is acceptable so as to introduce it to the urban people.

Scale:-
Y-axis: 10mg



AVERAGE INITIAL AND FINAL CALCIUM CONTENT
OF FEMUR AND TIBIA OF ALBINO RATS,
ON BASAL AND EXPERIMENTAL DIETS.

FIGURE 18

The food preparations chosen for conducting the palatability tests were 'Porial and Maseel' (refer Table II on page 49)

The test was carried out thrice, and the results of such tests are given in Table XI, presented below.

TABLE XI.

TOTAL SCORE OBTAINED BY DIFFERENT PREPARATIONS FROM
AMARANTH AND KALAVAI KEERAI.

Preparations	Colour	Texture	Taste	Appearance	Flavour
Amaranth.					
1. Maseel	38	36	45	41	49
2. Porial	46	38	49	48	47
Kalavai Keerai.					
1. Maseel	39	38	46	43	50
2. Porial	48	37	48	49	48

Table XI shows the different scores obtained by different preparations such as Maseel and Porial of amaranth and Kalavai keerai respectively. As it is seen, both the preparations of the respective greens have obtained more or less similar scores. Therefore it is inferred that Kalavai Keerai is as good as amaranth with regard to palatability, and hence the investigator is of the opinion, that this valuable Kalavai Keerai, could be accepted in the urban areas without any difficulty.

V SUMMARY AND CONCLUSION

The comparative retention of calcium from ~~amaranth~~ (common leafy vegetable of urban area) and kalavai keerai (common leafy vegetable of rural area) was studied, using weanling albino rats, ~~comparing~~ both the leafy vegetables at iso calcium level, to poor rice diet, which would supply calcium at the optimum level for the rats, that is 50 mg. per day according to Farris (1959), which is equivalent to 1.5 gm. of ~~amaranth~~ and 2.6 gm. of kalavai keerai respectively, in the dehydrated form. The availability of calcium from these two leafy vegetables was judged by using the following ~~criteria~~ growth, calcium balance and calcium in bone ash.

Kalavai keerai as a supplement, enhanced the growth rate of rats when compared with the control rats, and the difference was found to be significant. Data regarding the calcium ~~retention~~ by the calcium balance study and calcium in bone ash study also revealed the fact, that in both cases, kalavai keerai as a supplement stands superior to ~~amaranth~~ with highly significant differences.

From the analysis of the nutrient content of kalavai keerai it was noted to be a good source of minerals and carotene. The palatability test also points out that kalavai keerai is as palatable as ~~amaranth~~, since it has obtained more or less similar total scores as that of ~~amaranth~~.

Kalavai keerai is a good source of minerals and vitamins, and is superior to amaranth with regard to growth and calcium retention when supplemented to the poor rice diet, and can form an inexpensive source of calcium for the South Indian rice diets, since its palatability is as good as amaranth.

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APPENDIX I.
INTERVIEW SCHEDULE.

I.

1. Name of the head of the family:-
2. Serial No:-
3. Door No:- Street:-

II. Types of greens used, most commonly in the order of priority:-

- 1.
- 2.
- 3.
- 4.
- 5.

III. Frequency of the Purchase:-

IV. Most common and frequent method of preparing greens:-

V. Prevailing notions, regarding the inclusion of greens in the diet:-

- 1.
- 2.
- 3.
- 4.
- 5.

APPENDIX II.

SAMPLE CARD, USED ON THE CAGES OF RATS.

Expt., No.	Cage No.	Diet:-	Cardi-		
Date	Weight (grams)	Food given (grams)	Food left over (grams)	Food eaten (grams)	Remarks.

APPENDIX III.

SCORE CARD FOR PORIAL AND MASAL.^S

Instruction: Please tick against the appropriate criterion, under each quality.

Name:-

Date:-

Criteria.

~~Assessment~~ ~~Assessment~~
Porial Masal Porial Masal.

1. Colour.

- a. Natural Colour
- b. Olive green
- c. Dark green

2. Doneness.

- a. Well cooked
- b. Under cooked
- c. Over cooked

3. Appearance.

- a. Good
- b. Fair
- c. Poor

4. Taste.

- a. Good
- b. Fair
- c. Poor

-
- a. Scores 3 marks in all the qualities.
 - b. Scores 2 marks
 - c. Scores 1 mark

APPENDIX V.

CALCIUM INTAKE AND PERCENTAGE RETENTION IN ALBINO RATS.

Diets.	THIRTY WEEK.						THIRTY WEEK.						
	Mean Ca. intake per day mg.	Mean Ca. in faeces per day mg.	Mean Ca. in urine per day mg.	Total Ca. excreted mg.	Balance	percentage retention	Mean Ca. intake per day mg.	Mean Ca. in faeces per day mg.	Mean Ca. in urine per day mg.	Total Ca. excreted mg.	Balance	percentage retention	Mean percentage retention
Basal diet	1.8	1.8	2.0	3.8	- 2.5		2.6	2.2	2.0	4.2	-1.6		
" "	1.8	1.9	1.9	3.8	- 2		2.4	2.1	2.2	4.3	-1.9		
" "	1.9	2.1	1.8	3.7	- 1.8		2.4	2.1	2.0	4.1	-1.7		
" "	242.0	2.2	2.0	4.2	- 2.2		2.7	2.5	2.4	4.7	-2.0		
Basal + seaweed	27	12	7	19	8	29.6	31	15	8	23	8	25.8	
" "	25	14	6	20	5	20.0	29	13	7	20	9	31.0	25.6
" "	28	13	5	18	10	34.3	32	14	9	24	8	25.0	
" "	30	15	6	21	9	30.0	34	16	9	27	7	20.5	
Basal + Zilaival													
Leavel	24	11	5	14	10	41.5	30	13	9	22	8	26.5	
" "	29	13	4	17	12	41.3	29	11	6	17	11	38.0	34.5
" "	26	9	5	14	12	46.1	33	14	7	21	12	36.3	
" "	28	11	4	15	13	46.4	36	15	8	23	13	36.0	

APPENDIX IV.

GAIN IN WEIGHTS OF RATS DURING THE EXPERIMENTAL PERIOD (8 WEEKS)

Diets.	Initial		1st week		2nd week		3rd week		4th week		5th week		6th week		7th week		8th week	
	Wt. gm.	Mean gram.	Wt. gm.	Mean gram.	Wt. gm.	Mean gram.	Wt. gm.	Mean gram.	Wt. gm.	Mean gram.	Wt. gm.	Mean gram.	Wt. gm.	Mean gram.	Wt. gm.	Mean gram.	Wt. gm.	Mean gram.
Basal diet.	55.7	—	67.5	—	70.5	—	73.0	—	75.7	—	80.2	—	84.6	—	88.0	—	90.5	—
" "	65.5	69.0	54.4	63.0	56.9	65.7	63.4	66.5	62.9	69.6	61.7	71.9	70.6	77.8	74.6	80.2	77.2	85.2
" "	49.5	—	50.7	—	61.2	—	65.4	—	68.4	—	64.5	—	70.0	—	67.2	—	71.5	—
" "	60.0	—	68.6	—	73.1	—	75.2	—	80.2	—	81.5	—	85.0	—	93.0	—	100.6	—
Basal + guaranth	43.5	—	57.5	—	59.0	—	60.8	—	64.9	—	71.0	—	79.9	—	85.8	—	92.0	—
" "	50.2	48.4	34.7	64.1	67.6	68.1	70.7	67.9	73.9	75.4	79.6	80.2	95.5	89.9	81.8	96.1	95.8	101.7
" "	57.5	—	71.1	—	75.4	—	77.1	—	82.7	—	80.5	—	101.5	—	107.9	—	116.0	—
" "	46.5	—	63.2	—	64.5	—	65.6	—	72.3	—	80.5	—	91.9	—	94.9	—	104.0	—
Basal + Valisval Keorol	50.2	—	64.0	—	65.2	—	65.8	—	69.6	—	71.6	—	79.5	—	86.7	—	87.6	—
" "	45.5	49.0	35.6	64.6	60.2	67.0	61.8	68.9	69.9	71.9	70.7	82.7	80.1	81.4	93.9	88.2	98.2	104.7
" "	49.7	—	62.1	—	65.5	—	68.9	—	74.9	—	85.1	—	91.8	—	106.0	—	108.5	—
" "	51.0	—	74.1	—	77.5	—	79.5	—	84.9	—	82.9	—	104.2	—	112.9	—	119.1	—

APPENDIX. VI.

CALCIUM CONTENT OF FEMUR AND TIBIA OF ALBINO RATS ON
BASAL AND EXPERIMENTAL DIETS.

Diets.	Initial Ca. con- tent. mg.	Mean mg.	Final Ca. con- tent. mg.	Mean mg.	Net Ca. con- tent. mg.
1. Basal diet	18.0		20.3		
2. " "	18.5	18.2	20.2	20.2	2.0
3. " "	18.3		20.0		
4. " "	18.2		20.4		
5. Basal + Amaranth	18.0		42.3		
6. " "	18.5	18.2	41.7	42.6	24.4
7. " "	18.3		43.3		
8. " "	18.2		43.4		
9. Basal + Kalavai Keerai	18.0		48.9		
10. " "	18.5	18.2	49.9	49.4	31.2
11. " "	18.3		48.5		
12. " "	18.2		50.6		